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CARBOHYDRATE ABSORPTION BY ROOTS OF *PINUS TAEDA*.

By H. E. YOUNG, D.Sc.Agr., Pathologist, Science Branch, Division of Plant Industry.

SUMMARY.

A laboratory experiment demonstrated that mycorrhizal fungi are able to produce soluble carbohydrate from cellulose in sufficient quantities to support the growth of seedlings of Pinus taeda in sand culture in the absence of photosynthesis, and also that maltose in the absence of mycorrhizas can, as a soil nutrient, substitute for photosynthetic activity to some extent.

INTRODUCTION.

The part played by organic matter in the soil in the occurrence of fused needle-disease of *Pinus* was dealt with in a previous paper by the author (Young, 1940a), and observations made in South Africa by Sherry (1941) emphasized the importance of organic matter in pine nutrition.

In order to obtain further information concerning the rôle of organic matter in pine nutrition, experiments were designed and carried out with the object of determining whether carbohydrates obtained from insoluble cellulose material can be made available to pine plants with the aid of their mycorrhizal fungi. Seedlings of *Pinus taeda* were used as test plants. An attempt was made to grow seedlings of this species under aseptic conditions in a carbon-dioxide-free atmosphere with various sources of carbohydrate supplied in the rooting medium. From this it was hoped that evidence concerning the carbohydrate relationships of pine roots would be obtained.

PRELIMINARY TESTS.

Prior to proceeding with the main experiment a reducing sugar was shown to be present in healthy mycorrhizas and absent from non-mycorrhizal roots.

A further preliminary test was carried out which involved the growth of *Boletus granulatus* and *B. viscidus* on pure cellulose. Both these species of fungi form mycorrhizas with *Pinus* (Young, 1940b). *B. granulatus* is one of the

most common mycorrhiza formers in Australia and is plentiful in Queensland. The cellulose (filter paper) was cut up and sterilized in Erlenmeyer flasks after the addition of a carbohydrate-free nutrient solution consisting of—

Potassium dihydrogen phosphate	0·1	gm.
Magnesium sulphate	0·169	gm.
Calcium chloride	0·054	gm.
Colloidal iron phosphate	0·1	ml.
Water	1·0	litre

Tests with Fehling's solution for the presence of reducing sugars in the medium were negative. The paper alone also gave a negative test.

After sterilization the flasks were inoculated with pure cultures of the two fungi concerned. The least possible amount of inoculum, which consisted of a small quantity of aerial hyphae in each case, was used. Ten flasks were inoculated with each species of fungus. The cultures were then incubated at 27° C. At the end of a fortnight samples from all flasks gave positive tests with Fehling's solution, and quantitative determinations of the reducing sugars in all cases in the remaining material were made in the chemical laboratory.

The experiment demonstrated that the two fungi—*B. granulatus* and *B. viscidus*—are able to digest cellulose and in the course of the process produce a reducing sugar. This supported the previously stated hypothesis (Young, 1940a) that the mycorrhizal fungi of *Pinus* are able to use cellulose which is supplied in forest litter and in the course of its decomposition produce soluble carbohydrates which may be used by the higher plant. The importance of the presence of fresh supplies of organic matter is shown by the fact that only in the unrotted condition is there an appreciable quantity of cellulose available.

It was also indicated that the growing of *Pinus* in a carbon-dioxide-free atmosphere might be possible.

MAIN EXPERIMENT.

In order to test the possibility of growing *Pinus* in a carbon-dioxide-free atmosphere an experimental design was devised whereby seedlings of *P. taeda* could be kept without carbon dioxide and their source of nutrients controlled. Three sets of conditions with respect to nutrient supply were imposed, viz.,

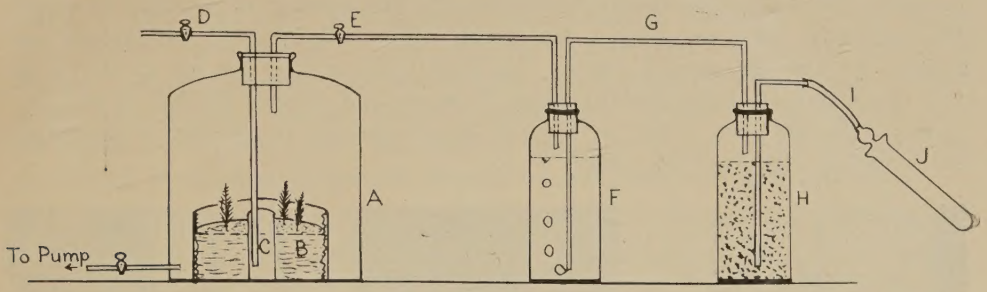
(1) Sand in glass dishes plus chopped filter paper (20 gm.) and carbohydrate-free nutrient solution as given on page 2.

(2) Sand, filter paper, carbohydrate-free nutrient solution and maltose (20 gm. per litre).

(3) Sand, filter paper, carbohydrate-free nutrient solution and inoculation with *Boletus granulatus*.

Filter paper as a source of available carbohydrate was common to all treatments.

The general design of the experiment will be seen on referring to the following diagram and description:—

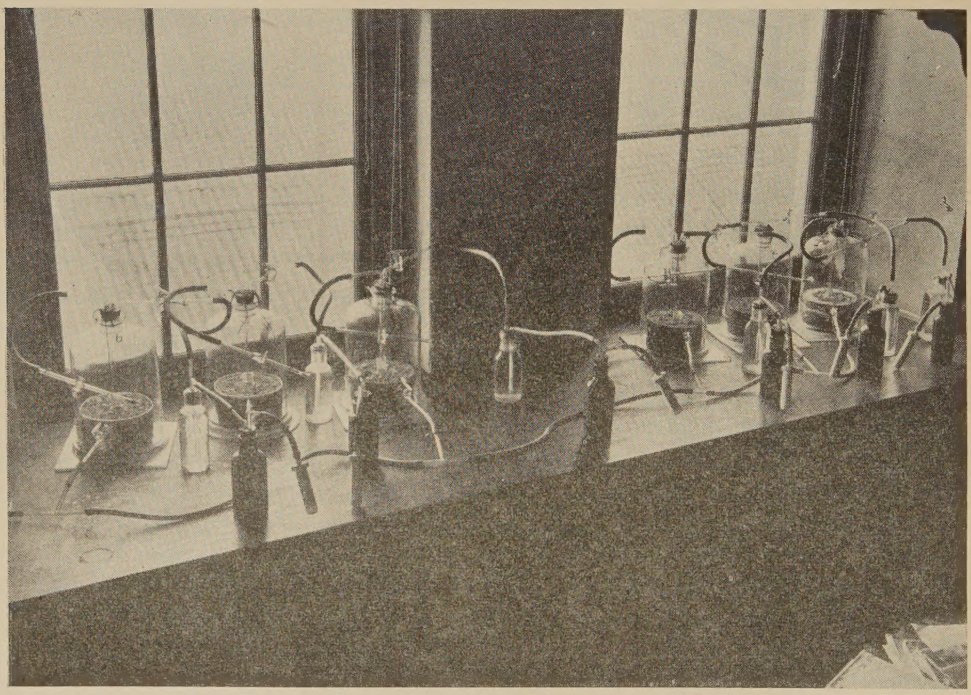


Diagrammatic representation of design of one unit of the carbohydrate experiment.

The bell jar A covered the glass dish B, which was 4½ inches deep and 7 inches in diameter and contained washed (acid and alkali then distilled water) river sand as a rooting medium. In the sand was placed a 1-inch diameter glass tube (C) with a gauze apron tied around the bottom. The tube had a serrated bottom in order to allow liquid to percolate in and out of the tube. The gauze was used to prevent sand from moving into the glass well C and interfering with the flow of fluids. A glass tube with a tap was fitted into the rubber stopper of the bell jar and arranged so that it came within half-an-inch of the bottom of the well C in the sand. A second glass tube with stopcock was arranged so as to pass a short distance through the stopper. This tube was connected by rubber pressure tubing to a bottle (F) by means of a glass tube just penetrating the stopper. This bottle contained a 5% solution of fresh sodium hydroxide plus 50 ml. of saturated solution of barium chloride, yielding baryta water. Another tube (G) was placed in the bottle so as to pass through the stopper to within half-an-inch of the bottom of the bottle and from this bottle by means of pressure tubing and a short glass tube through the stopper of the second bottle (H). This second bottle contained granulated soda lime. From H a glass tube (I) passed almost from the top up through the stopper and was connected by pressure tubing to a Berkfield-Chamberlain bacteria-proof porcelain filter (J). Through the side of the bell jar (A) as a means of exhausting the apparatus a glass tube with a stopcock was passed through a rubber stopper so as to reach just inside it. The other end of the tube was connected by pressure tubing to the filter pump. This exhaust tube was placed near the bottom of the apparatus in order to remove any carbon dioxide which might accumulate.

The object of the apparatus was to provide a means of aerating the bell jar with carbon-dioxide-free air. On operating the suction by means of the suction pump and opening all the stopcocks save that in the tube D, air was drawn through the filter and sterilized, then through the soda lime and freed of carbon dioxide, bubbled through the caustic soda-barium chloride solution and so into the bell jar and out into the pumping mechanism.

The baryta water solution acted as a check on the efficiency of the soda lime by producing a milky precipitate if and when any carbon dioxide got past the soda lime bottle. The rate of passage of air was governed by means of the pressure exerted by the suction pump and by the amount of opening to which the stopcocks were regulated. In practice it was run at three bubbles per second through the baryta water. Six such systems were set up as shown in the plate and all connected by glass T-pieces and rubber tubing to the one pump. The six sets could be worked simultaneously or in any combination and could be disconnected easily.



Battery of six CO_2-free jars with pine seedlings growing in nutrient solution.
[*Photograph by E. Suchting.*

All joints were sealed with Gurr's mounting medium. The bell jars were affixed to their ground glass bases by boiled Vaseline which was finally coated with euparol on the outside of the joints.

The apparatus was set up on a laboratory bench with no direct sunlight and arranged so that all bell jars received as nearly as practicable the same amount of illumination.

During the course of the experiment room temperatures varied from 70° to 79° F.

The complete apparatus was steam sterilized, six germinating seeds of *Pinus taeda* were placed in each glass dish under aseptic conditions, and the apparatus was closed and sealed.

Difficulty was experienced in obtaining sterile seed. This was overcome by removal of the testa and sterilization of the seed surface for 20 minutes in bleaching powder solution (10 gm. in 140 ml. water). The seeds were then germinated on potato dextrose agar in sterile Petri dishes. A large number of seeds was germinated in this way and when sufficient sterile seeds had radicles about 1 inch long they were planted out. Aseptic precautions were taken throughout. In treatment 3, involving the mycorrhizal fungus, the dishes at the time of seed planting were seeded with an inoculum of a fresh pure culture of the mycorrhizal organism, *Boletus granulatus*. (When *Boletus viscidus* was used as an inoculum it proved too vigorous and smothered the seedlings under the conditions of the experiment; *B. granulatus* was eminently satisfactory.)

For the first 10 days ordinary sterilized air was pumped through the apparatus continuously but without passing through the soda lime and baryta water. At this time the cotyledons of the seedlings were visible. After this initial period carbon-dioxide-free air, obtained by placing the soda lime and baryta water bottles in the circuit, was used. By continuous pumping, the carbon dioxide in the bell jars at the end of the initial period and subsequently any carbon dioxide respired by the mycorrhizal fungi on the plants was removed. Periodic checks for carbon dioxide in the exhausted air were made with the aid of baryta water.

Difficulties due to contamination of the dishes by foreign organisms were experienced but finally overcome. These contaminations occurred at the planting of the seed and were usually associated with the seed itself.

At 21-day intervals the dishes were freshly irrigated with their respective sterile nutrient solutions and then drained again. This was effected by passing sterile nutrient solution into the dishes by means of the irrigating tube D. The flow was induced by manipulating the stopcocks and drawing the fluid in by means of the suction pump. The dishes were flooded and the excess solution then removed by means of the glass wells (G), which allowed any free solution to be siphoned from the sand in the dishes back into the flasks of nutrient solution.

Observations.

The appearance of the seedlings as brought about by each of the treatments was as follows:—

1. *Soluble carbohydrate-free, mycorrhiza-free nutrient.* Seedlings were spindly, grey-yellow-green in colour; the cotyledons were reflexed and the plants gradually wilted. No primary needles developed. The seedlings soon lost the power of supporting their own weight and finally shrivelled and died. Death commenced at the cotyledon tips. Mean life was 59 days.

2. *Maltose added—no mycorrhizal fungus.* Good growth but not so vigorous as 3. Plants became marked by punctate depressions on the cotyledons and primary leaves, which were somewhat distorted. This was possibly due to too high a concentration of the nutrient solution. Growth gradually ceased. Primary leaves appeared at 29 days. Mean life was 102 days, with one plant still living at 140 days, but with poor vigour. The roots of the dying plants

persisted in the living state for a considerable period after the death of the tops. The behaviour of these sugar-fed plants accords with the results obtained by Knudson and Lindstrom (1919), who attributed the poor growth of albino corn plants in a nutrient containing carbohydrate to the slow diffusion of the sugar. For this reason the plants were unable to absorb a sufficient quantity of organic material to supply the needs of the entire plant, the roots alone being sustained. The presence of mycorrhizas is not mentioned by the authors and it is, therefore, assumed that they were absent.

3. *Soluble carbohydrate-free; mycorrhizas present.* Good growth; some deaths from smothering by overgrowth of the mycorrhizal fungus. Primary leaves were produced in 21 days, that is eight days before those plants treated with carbohydrate in the absence of fungi. Mean life was 270+ days. Six plants were still growing vigorously with no signs of decline at the time of completion of the experiment. The remainder were smothered by fungus growth in their early stages. Mycorrhizas were present on the roots of the seedlings.

The experiment was repeated and the observations on the second series agreed with those on the first series as described. As each treatment was duplicated in each series, four replications of each treatment were carried out with similar results. In the second series the aeration treatment was modified to some extent. It was discontinuous and carried out only for one hour per day for the first 42 days and then continuously again. This was done because of inability to obtain adequate supplies of soda lime owing to wartime difficulties. This was regrettable, since fungal respiration of carbon dioxide in the mycorrhiza-inoculated jars would not be continuously eliminated. However, the two series corresponded in their reactions and with the resumption of continuous aeration no change was noted, showing that this factor was not serious.

CONCLUSIONS.

The experiment showed that seedlings of *Pinus taeda* can exist and grow in a carbon-dioxide-free atmosphere and that the fungus *Boletus granulatus* is capable of providing pine roots with a carbohydrate supply derived from cellulose and suitable for pine nutrition. Maltose supplied in solution also supports growth much longer than sugar-free media but not for so long and not so well as that provided by mycotrophic means.

It was also shown that mycorrhizas with the aid of organic matter are able to supply carbohydrate to the plants by means of the roots and that this supply can be at least accessory to that supplied by photosynthetic activities.

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STORED PRODUCT PESTS IN NORTHERN QUEENSLAND.

By N. E. H. CALDWELL, M.Sc.Agr., Horticulturist.*

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E.M.N.

SUMMARY.

A survey of stored product pests in northern Queensland was made in 1942 and 1943 and supplementary information was gathered at intervals up to the end of 1945. Most attention was devoted to foodstuffs for human consumption.

Thirty-four pest or potential pest species were recorded. The relative importance of all species at the time of the survey is indicated, but it is pointed out that under normal peacetime trading conditions this relationship may change to some extent. Tribolium castaneum Herbst. and Corcyra cephalonica Staint. were by far the most important, followed by Calandra oryzae L., Oryzaephilus surinamensis L. and Ephestia cautella Walk.

The recognition of Corcyra cephalonica as a major general pest, at least in northern Queensland, and of T. castaneum as an important pest of finely milled cereal products as well as many other commodities, were the outstanding findings of the survey.

INTRODUCTION.

During 1942 and 1943 a survey was made of stored product pests in northern Queensland and interest in the problem was maintained until the end of 1945. Attention was concentrated on the important foodstuffs for human consumption; but, when opportunity offered, the insect fauna of other human foodstuffs, stock foods and other commodities and of factory and storage premises was investigated.

The survey was undertaken during a period when conditions were abnormal because of the war. A number of commodities normally in common use either were in very short supply or had disappeared entirely from ordinary trade channels. Also, in many cases, storage arrangements, which can have considerable influence on insect infestation, were not typical of peacetime commerce. Thus it could be expected that under normal trading conditions the relative importance of some of the species would be altered to some extent. In addition, further attention to stock foods, agricultural seeds, &c., would no doubt provide supplementary information which may also change the general picture in some particulars.

The territory covered in the survey extended along the coast from Mackay in the south (approximately 21° S. lat., 149° E. long.), to Cairns in the North (approximately 17° S. lat., 146° E. long.), and as far inland as Richmond (approximately 21° S. lat., 143° E. long.). Four main climatic types were involved, namely wet coastal, dry coastal, wet subcoastal highland, and dry inland.

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TABLE SHOWING RELATIVE IMPORTANCE OF STORED PRODUCT PESTS IN NORTHERN QUEENSLAND.

Insect.	Whole Grains.	Crushed and Cracked Grains.	Milled Cereal Products.	Processed Cereal Products.	Leguminous Seeds and Seed Products.	Dried Fruits.	Nuts and Nut Meats.	Tobacco and Other Dried Plant Products.	Dried Meats.	Factory and Warehouse Debris.
COLEOPTERA.										
FAM. TENEBRIONIDAE.										
Tribolium castaneum Herbst. ..	++	++++	+++++	++++	..	..	++	..	..	++++
Tribolium confusum J. du V. ..	+	+	++	..	..	..	..	..	..	+
Gnathocerus cornutus F.	+	..	++	..	..	..	..	..	..	++
Palorus subdepressus Woll.	+	..	..	..	..	..	..	..	..	+
Latheticus oryzae Waterh.	..	..	+	..	..	..	..	..	..	..
Alphitobius piceus Oliv.	..	..	+	..	..	..	..	..	..	+
Unidentified species	..	..	..	..	..	..	..	..	..	++
FAM. CURCULIONIDAE.										
Calandra oryzae L.	++++	++	+	++	..	..	..	..	..	+++
FAM. BOSTRYCHIDAE.										
Rhizopertha dominica F.	++	++	++	+	..	..	..	+	..	+
FAM. CUCUJIDAE.										
Oryzaephilus surinamensis L. ..	+	+	++	+++	..	+++	+++	+	..	++
Oryzaephilus unidentatus F.	..	..	+	..	..	..	..	..	..	..
Laemophloeus minutus Oliv. ..	++	++	++	..	..	..	..	..	..	+
FAM. TROGOSITIDAE.										
Tenebroides mauritanicus L. ..	+	..	++	..	..	..	..	..	..	++
Lophocateres pusillus Klug.	..	..	..	..	..	..	..	..	..	++
FAM. PTINIDAE.										
Lasioderma serricorne F.	..	+	++	..	..	..	+	++	..	+
Stegobium paniceum L.	..	..	++	++	..	..	..	++	..	..
Gibbium psylloides Czemp.	..	..	+	..	..	..	..	..	..	..
FAM. BRUCHIDAE.										
Bruchus obsoletus F.	..	..	..	..	++	..	..	..	..	..
Bruchus quadrimaculatus F.	..	..	..	..	++	..	..	..	..	..

FAM. NITIDULIDAE.											
Carpophilus dimidiatus F.	+	+	++	..	..	..	..	..	..	..	
Carpophilus hemipterus L.	..	..	+	..	..	..	..	..	..	..	
Carpophilus fusus Man.	..	..	+	..	..	..	..	..	..	..	
Carpophilus sp.	..	..	+	..	..	..	..	..	..	..	
FAM. DERMESTIDAE.											
Trogoderma sp.	..	..	+	..	..	..	..	..	..	+	
Dermestes sp.	..	..	..	..	..	..	..	..	+	..	
LEPIDOPTERA.											
FAM. GALLERIIDAE.											
Corcyra cephalonica Staint.	+++	+++	+++++	++++	++	+	++	+	+	++++	
FAM. PHYCITIDAE.											
Ephestia cautella Walk.	+	++	+++	++	++	++	−−	−−	−−	++	
Ephestia kuhniella Zell.	−−	−−	++	−−	−−	−−	−−	−−	−−	++	
Plodia interpunctella Hbn.	..	..	++	++	..	++	++	..	..	+	
FAM. GELECHIIDAE.											
Sitotroga cerealella Oliv.	++	..	..	..	..	..	..	..	..	++	
THYSANURA.											
FAM. LEPISMATIDAE.											
Ctenolepisma longicaudata Esch. ..	..	..	..	..	..	..	..	..	..	++	
COPEOGNATHA.											
FAM. TROCTIDAE.											
Troctes divinatorius L.	++	..	++	..	++	..	..	..	..	++	
ACARINA.											
FAM. CALOGLYPHIDAE.											
Thyraeophagus entomophagus Lab. ..	..	..	++	..	..	..	..	..	..	..	

PEST SPECIES.

The pest species encountered are listed in the accompanying table and an attempt is made to indicate their importance in various classes of stored products. In making this assessment, distribution throughout the area of survey, frequency of occurrence in any class of product and the population density usually found, were all taken into account. Thus a species widely distributed, frequently associated with the product and usually with a high population density is given the rating +++++, e.g., *T. casteneum* in milled cereal products. A species found only in numbers so small as to be of no practical importance is given the reading +, e.g., *C. cephalonica* in dried meats. Intermediate degrees of importance are shown by ++, +++, and ++++.

DISCUSSION.

The table shows that *Tribolium castaneum* and *Corcyra cephalonica* are the most important species. Because of the large size of the larva and its web-spinning habit, the latter is popularly regarded as the more serious pest, but in fact it probably does not contaminate a greater quantity of foodstuffs.

C. cephalonica has not been recognized previously as a major stored product pest in Queensland. It has not even yet been recorded from southern parts of the State, though its distribution elsewhere suggests that conditions in the south are not unfavourable to it. If present, it must therefore be of only incidental importance. The very wide range of materials attacked in the north should be noted.

T. castaneum is generally regarded more as a secondary pest of whole grains than as a primary pest of finally milled cereal products. However, in northern Queensland it reaches its maximum development in the latter capacity. The comparative scarcity of the closely allied *T. confusum* is of interest. Climatic conditions would not appear to offer any barrier to its successful establishment, as, when it did occur, populations were often large and thriving laboratory colonies were maintained for many months at a dry coastal station. The closely related *Gnathocerus cornutus* is given an equivalent ranking to *T. confusum*. It is, however, somewhat more widely distributed but, on the other hand, populations do not attain the density of those of the latter species.

Next in order of importance to *T. castaneum* and *C. cephalonica* are *Calandra oryzae, Oryzaephilus surinamensis* and *Ephestia cautella.*

The status of *C. oryzae* may well be increased when a more detailed study is made of whole grains and other favoured breeding materials for this species. Population samples from various sources were examined for strain differences; only the large strain was found. It is interesting to note that the companion species *C. granaria* was not recorded.

Oryzaephilus surinamensis and *Ephestia cautella* may increase in importance when certain commodities, notably dried fruits, nuts and nut meats, herbs, spices, confectionery, &c., circulate freely again. *Plodia interpunctella, Stegobium paniceum* and *Lasioderma serricorne* would also be expected to play a more important part in the contamination of such products.

Bruchus spp. are probably of somewhat greater importance than indicated as pests of leguminous seeds for agricultural purposes. Similarly *Sitotroga cerealella* may occur more commonly as a pest of maize, particularly in the field. Beetles of the family *Dermestidae* probably occur commonly in association with certain products of animal origin, such as hides and skins.

The unidentified species of *Tenebrionidae* was fairly prevalent in debris associated with grain crushing machinery at one centre. A pure colony was maintained in white flour in the laboratory for some months but the rate of increase was small compared with that of *Tribolium* spp. Nevertheless, the beetle is presumably a minor pest.

Thyraeophagus entomophagus and perhaps other species of mites may be more prevalent than shown by this survey, as when special methods of examination were not used many cases of mild infestation may well have passed undetected. However, development of the characteristic "musty" odour in such commodities as flour, due to gross infestation by mites, was not encountered and mite infestation therefore seems of minor importance in northern Queensland, at least under most conditions.

The superiority of wholemeal flour to white flour as a breeding medium for many species is thought to be of little practical importance in northern Queensland. Large populations of certain species, notably *T. castaneum* and *C. cephalonica,* will quickly develop in white flour in the tropics. Initial contamination, the risk of which must be greater in the case of coarse-ground materials, is believed to be a more important factor governing rate of deterioration due to insect infestation.

There is obviously no relation between distribution and population densities of the major pest species and the main climatic zones of the area surveyed. Sufficient evidence is not available to indicate if climate has any influence on distribution or population levels of the less prevalent species.

ACKNOWLEDGEMENTS.

Thanks are due to Dr. A. Jefferis Turner, of Brisbane, for his identification of some of the Lepidopterous species; to Mr. L. C. Birch of the Waite Agricultural Research Institute, Adelaide, for his examination of *Calandra oryzae* specimens to determine strain differences; and to several members of the Head Office staff of the Queensland Department of Agriculture and Stock for checking the identity of all other specimens.

MALIGNANT OEDEMA OF LAMBS IN NORTH-WESTERN QUEENSLAND.

By A. K. SUTHERLAND, B.V.Sc., and G. R. MOULE, B.V.Sc., Veterinary Officers, Division of Animal Industry.

SUMMARY.

Heavy mortalities in lambs after marking are shown to be due to infection by Clostridium septique through the scrotal wounds.

Effective control may be achieved by carrying out lamb-marking operations in temporary yards.

INTRODUCTION.

There is no dearth of information on anaerobic infections of domestic animals, but there are not many records in Australian veterinary literature of heavy mortalities in lambs due to *Clostridium septique* infection of castration wounds.

Scott, Turner and Vawter (1935) classified malignant oedema as a wound infection and this appears to be the accepted opinion. Edgar (1931) recorded mixed anaerobic infections following crow pecks of sheep in New South Wales. Borthwick (1943) studied factors which influenced infection by *Cl. septique* and he concluded that reduction of the activity of the alimentary tract, and to a less extent exposure to cold, predispose to infection through the alimentary tract. Steward (1944) described malignant oedema in swine probably due to *Cl. septique* but was unable to determine the mode of infection in all cases. He suspected an alimentary route in six of the nine outbreaks recorded and considered that inoculation with serum or vaccine appeared to be a factor in precipitating some *Cl. septique* infections.

Oxer (1935) stressed the importance of fatness in predisposing sheep to black disease and entero-toxaemia and he is of the opinion (Oxer, 1947) that fatness predisposes to anaerobic infections generally. Bosworth (1943) stated that *Cl. septique* is frequently present in the intestinal canal of healthy animals. Kranveld and Djaenoedin (1940) reported that *Cl. septique* is often present in the bile of healthy cattle slaughtered in Java. Seddon and Edgar (1930) surveyed the occurrence of anaerobes and found *Cl. septique* in 28 per cent. of samples obtained from dairy yards. Of those obtained from cultivated (wheat) lands 20 per cent. were contaminated by *Cl. septique*, whilst the organism was recovered from only 8 per cent. of the soil samples obtained from grazing land. *Cl. septique* was not demonstrated in any of the soil samples obtained from completely virgin soil, i.e. from land that was completely uncultivated and ungrazed.

An investigation was made by one of us (G.R.M.) into mortality in young rams on a property between Winton and Hughenden in 1942. The animals died on being unloaded after a long train journey, and the symptoms and post-mortem findings were consistent with a *Cl. septique* infection. This was confirmed by laboratory examination but the route of infection was not established.

A mortality in rams was investigated by one of us (G.R.M.) in central-western Queensland in 1943. Four hundred rams had been culled from a stud when they were about 14 months old and were castrated at a set of permanent yards. The scrotum was slit and the testicles removed by an emasculator. About 40 animals had died within six days and another 50 seemed likely to die. Laboratory examination of specimens by one of us (A.K.S.) revealed *Cl. septique* to be present and the symptoms and post-mortem lesions were consistent with a *Cl. septique* infection.

Losses in lambs after marking (i.e. castration, docking and earmarking) have been reported on several occasions in Queensland. The common sequelae previously recorded have been blowfly strike, suppurative and non-suppurative polyarthritis due to *Corynebacterium pyogenes* and *Erysipelothrix rhusiopathiae* respectively, tetanus and shock (Edgar, 1931). In addition, it has been known for some years that heavy mortalities (from 10 per cent. to 50 per cent.) have occurred in recently castrated male lambs in north-western Queensland and the circumstances suggested that an anaerobic infection was responsible.

In north-western Queensland lambing percentages (i.e. percentage of lambs marked to ewes mated) are very low, 50–60 per cent. being considered a good figure while the average is in the vicinity of 35–40 per cent. In addition many years occur when seasonal conditions are so bad that it is not possible to mate the sheep at all. The summer rains, which predominate, are very unreliable. Hence the usual practice is to mate when the rains fall and there is assurance of 5 to 6 months of feed (mainly Mitchell and Flinders grasses) rather than to mate in anticipation of rain and good feed for lambing. As prolonged periods of hot weather occur during summer (Kynuna for instance having 6 consecutive months with average daily maxima over 95°F.), it seems likely that the fertility of the rams would be low (Gunn *et al.*, 1942). In addition, mating after the first heavy summer rains means lambing when the ewes are on a falling plane of nutrition, and when much of the surface water is drying up so that the pregnant ewes or the ewes and their young lambs have to travel long distances over open shadeless downs to water. Under such conditions the wastage of both lambs and breeding ewes is high and mortalities in lambs which have been raised to marking age assume considerable importance.

The cases described in this paper relate to heavy mortalities in lambs after marking. They occurred in north-western Queensland during the winters of 1944 and 1945. At least a dozen properties in the Richmond-Maxwelton districts were affected and on many of them the deaths exceeded 25 per cent. of all lambs marked. The trouble was confined to male lambs and usually the fattest and best grown were affected. Deaths commenced about 3 days after marking, were heavy on the 4th, 5th and 6th days and subsided after the 7th day. Gas gangrene developed in the inguinal region and there was marked lameness in the hind limbs prior to death.

In 1944 one property reported losing 1,500 lambs from 5,700 marked. Another lost 1,200 from 6,400 and another 783 from 4,800 marked. One owner reported marking 3,500 lambs just after the July rains of 1944. The sheep were

handled in five lots with one day between each marking. No trouble occurred amongst the first lot, but three died from the second, about 35 from the third, over 200 from the fourth, and out of the fifth, which comprised 845 lambs, 400 died. The total loss was about 700 lambs. As about 50 per cent. of all lambs marked are males the losses amounted to almost half of this sex.

On most of the properties the flocks were depastured according to age, and the various groups were mustered independently for marking. The experience quoted above was typical in that no losses were recorded in the flocks first marked, but mortalities occurred in the lambs from the flocks subsequently handled and the severity of the outbreak increased with each succeeding lot of lambs.

Permanent yards were used for handling the sheep at marking time. The yards on one property were at least 45 years old. Some of the yards were watered well to lay the dust.

The trouble seems to have been most marked after the unseasonable winter rains which fell in both 1944 and 1945. The weather after these rains was, however, warm and sunny.

On all properties the usual method of castration was used, the scrotum being "tipped" in order to expose the testicles. On some properties a blowfly dressing or an "antiseptic" solution was applied to the marking wounds. Mortality was not associated with any specific dressing, nor with flocks in which no dressing was used.

INVESTIGATIONS.

An investigation was carried out at a property south of Maxwelton during the four days July 27–31, 1945, when losses were occurring under circumstances similar to those reported during the previous year.

History.

The seasonal conditions in 1945 were good. Heavy summer rains were followed by 218 points in May and 225 points on July 10.

Marking was commenced at the shearing-shed yards early in July and about 700 lambs were drafted and then earmarked, castrated and tailed.

The sheep were yarded in the afternoon and next morning the lambs were drafted into a "check pen," at one end of which the cradles were mounted. After marking, the lambs were released in a large yard where the ewes were held to allow "mothering." Thus the sheep were in the yards about 24 hours. No wound or fly dressing was applied and no losses occurred.

The marking was interrupted by a ram shearing and when this was in progress the July rains fell. Three hundred rams were worked through the yards, which were still damp.

Lambmarking was resumed on July 14, when a flock of 2,000 ewes with 59 per cent. of lambs was handled. The work was completed on July 16. When the flock was inspected four days later, 18 wether lambs were found dead in the paddock and over 50 were either sick or dying.

On July 21, a total of 284 lambs was marked, and when inspected four days later wether lambs were dying. In a short search of the paddock (6,000 acres) six days after marking 25 dead lambs were found. One sick lamb was taken for post-mortem examination.

On July 24, 550 lambs were marked and 24 dead and 23 dying wethers were seen three days later. The marking technique was varied with this flock in that the scrotum and the tail stump of the male lambs were sprayed with a phenolic solution after the castration and tailing operations. As might be expected, this treatment did not prevent subsequent mortality. The ewes were allowed into a small well-grassed lane beside the yards and were held there to allow mothering. On being released, the lambs ran straight through the large holding yard, which had previously been used for mustering, into the lane. On July 27, 700 lambs were marked under similar circumstances, 40 being found dead within three days. In all cases the lambs' tails were cut at about the same length, and the stump was just long enough to cover the tip of the vulva in ewe lambs. In the males the tails were of comparable length. The total deaths on this property in 1945 amounted to 800 lambs from 6,152 marked.

Symptoms.

Sick lambs from a flock which had been marked three days earlier were examined during the afternoon of July 27.

They segregated themselves from their mothers and did not graze or suckle. They stood about with back arched, head down and subsequently lay down in lateral recumbency. Some lambs lay quietly when approached and were easily caught. Others showed a remarkable degree of activity and it was difficult to catch them. These animals showed lameness in one or both hind legs and in some cases were unable to flex the limb.

There was a hot oedematous swelling of the inguinal region and scrotum. Oedema extended along the belly and past the prepuce, which was swollen and reddened. The skin of the groin and abdomen exuded a sanguineous serum which stained the wool covering these parts. The skin over the groin was desquamated and the wool from the belly plucked easily.

Temperatures of over 106°F. and respirations of over 120 per minute were recorded. The pulse (rate 80 per minute) was inclined to be bounding. Later the pulse rate rose to 200 per minute and the heart was tumultous. The conjunctival mucosae were injected.

Post-mortem Findings.

Three male lambs were examined about 30 minutes after death. They had been marked three and six days previously. In each the castration wound was closed, and the scrotum was enlarged and filled with dark clotted blood.

The wall was oedematous and purplish-red, and when cut a purplish serous fluid was exuded. There was a large oedematous swelling over the groin and along the belly. The skin over the whole of this area was discoloured and exuded a blood-stained serum. The skin inside the legs was desquamated.

In some cases the discolouration spread from the scrotum over the perineum to the base of the tail. The wound on the stump of the tail was healing satisfactorily. The skin of the abdominal wall was thickened and discoloured and the subcutis was oedematous and exuded a dark-purple serous fluid. The abdominal muscles were discoloured; their blood vessels were engorged and the fibres were swollen and friable. This gave a "soggy, water-logged" appearance.

The parietal and visceral peritoneum was inflamed and was marked with large splashy haemorrhages. The liver was parboiled, the spleen slightly oedematous and the lungs petechiated.

The pericardium was extremely thickened, measuring about three-eighths of an inch, and it contained deep-purplish clotted exudate. The heart was very enlarged and the surface vessels were engorged and tortuous. The epicardium and endocardium were petechiated; the myocardium showed blotchy discolouration and was friable. There was dark clotted blood in the ventricles and a frothy blood-stained discharge from the nose.

Where the discolouration extended to the muscles of the perineum the fibre bundles were enlarged, dark and friable.

Bacteriological Examination.

Films from the peritoneal surface of the livers of three lambs were stained by Gram's method. They showed large numbers of Gram-positive bacilli. Filaments up to 10 μ in length were present and some organisms contained an oval subterminal spore.

Sealed Pasteur pipettes of the exudate from the abdominal wall of one lamb and from the scrotum of another both yielded cultures of *Clostridium septique*.

Pipettes were cultured in a cooked-meat medium (incubated aerobically) and on ox-blood agar plates (incubated anaerobically). On the plates apparently pure cultures of clear round colonies surrounded by narrow zones of haemolysis were present after 24 hours' incubation. The cultures from both lambs had the morphological characters of *Cl. septique*.

Subcutaneous injection of 0·5 ml. of an 18-hour culture in cooked-meat medium killed guinea pigs (about 450 gm. weight) in 18 to 40 hours. The usual features of *Cl. septique* infection were noted, including the presence of long Gram-positive filaments on the peritoneal surface of the liver.

Four mice inoculated intraperitoneally with 0·2 ml. of culture died in 24 hours, whereas four mice inoculated with the same dose of culture plus 0·2 ml. of *Cl. septique* antitoxin (Commonwealth Serum Laboratories) showed no ill effects.

The infecting organism was thus identified as *Cl. septique*.

Control Measures.

Previous experience in the sheep industry indicated that heavy losses associated with lamb marking can be prevented by carrying out the work in temporary yards. Accordingly some temporary yards were erected in a corner of one of the shearing-shed paddocks and 615 lambs were marked on July 28. All instruments were boiled and the male lambs were castrated and tailed by one of us (G.R.M.). The scrotum was "tipped" in the usual fashion; two "Luck" lamb marking knifes were used for the work, one being immersed in a strong "Dettol" solution while the other was in use. The ewes were tailed by the owner's son, who followed the procedure he had adopted at the previous markings after which trouble had occurred. The tail length adopted for the ewes was long enough to cover the tip of the vulva and that for the wethers was comparable. On being released the lambs ran into the paddock to the ewes, which were held for mothering.

Three days after marking five of the wether lambs had died. The symptoms and lesions were identical with those already described. No further sick lambs were seen but when the sheep were moved about a week after marking 10 lambs were missing.

The same procedure was used subsequently to mark 986 lambs in temporary yards on another part of the property and 15 dead lambs were found during the week following marking. These losses were less than those usually experienced in Queensland after marking male lambs: the estimate of one of us (G.R.M.) is that on most properties in Queensland 2½–3 per cent. of male lambs die each year after marking.

DISCUSSION.

The occurrence of the disease in male lambs only and the nature of the lesions leave little doubt that the infection gained entrance through the scrotal wounds. The tail length adopted at marking was that which according to Johnstone (1944) is most likely to assist rapid and uncomplicated healing. This may have been a factor in preventing mortality amongst ewe lambs, leaving the scrotal wounds of the males as the portal of infection. On the other hand, tail wounds are probably not of a type suitable for the germination and growth of anaerobic bacilli.

As the lambs were not drafted from their mothers until the morning they were marked, they were not subjected to prolonged starvation. This may have been sufficient to reduce the activity of the alimentary tract; but the possibility of infection through this route seems remote, especially as the weather was not cold.

The work of Bosworth (1943) and of Seddon and Edgar (1930) and to some extent Kranveld and Djanoedin (1940) suggests that much-used stockyards are places most likely to be heavily contaminated with *Cl. septique* spores. The increasing severity of the losses as the marking proceeded and the almost complete cessation of trouble when the marking was done in temporary yards might be regarded as confirmatory evidence of this. Topley and Wilson (1936) state that

spores of *Cl. septique* are widely distributed in the few inches of topsoil. They also consider that the presence in the wound of kataphylactic agents such as calcium salts is necessary for the development of a gas-gangrene lesion following the introduction of spores. Thus the reduction of losses by marking in temporary yards may have resulted from the lack of kataphylactic substances in the soil about temporary yards or from a lighter infection of *Cl. septique* spores.

A comparative study of the contamination by *Cl. septique* of sheep yards and paddocks in various parts of Queensland has not been undertaken. The soils of western Queensland are considered to contain an adequate amount of calcium, but no figures comparing the calcium content of the soils of sheep yards and paddocks are available.

The apparent association between the losses and the winter rains is also of interest. Under the conditions of management practised winter rains ensure fat, well-grown lambs, and Oxer (1947) considers fatness predisposes animals to anaerobic infections.

The losses amongst lambs which were allowed to "mother" in the grassed lane suggest that infection may have been picked up prior to the animals being castrated. The severity of the losses already experienced on the property where the mortalities were investigated did not permit further markings being undertaken in the permanent yards for experimental purposes.

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